The Effects of Supplementing Essential Long Chain Fatty Acids to Ewes in Late Gestation on Offspring and Ewes

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Abstract

Multiple studies have shown improvements in performance of livestock due to supplementation of polyunsaturated fatty acids (PUFA). Other studies have shown an effect of PUFA on fetal programming of offspring in livestock, though little work in this area has been done with ruminants. This research was conducted to evaluate the performance effect of ewes and their lambs when the ewes were supplemented with increasing concentrations of the PUFA docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as Ca salts during the last 50 d of gestation. The PUFAs were supplemented at concentrations of 0%, 1% and 2% of the diet fed to gestating ewes. Ewes (n=24 per treatment) started receiving the supplements 50 d prior (d -50) to expected lambing (d 1). Supplementation finished on lambing day and all ewes received the same diet after lambing. Ewes were weighed, and body condition scored (BCS) at d -50, d -20, d 15 and d 60 (weaning). On d 15 milk production and composition were evaluated after 3 hours of separation of the lamb from the ewe. Lambs were blood sampled (to measure plasma concentration of glucose and NEFA) and weighed on d 1, d 15, and d 60. Data were analyzed using a mixed model of SAS, using a linear and quadratic polynomial contrast for mean separation. There was a quadratic effect ($P = 0.01$) for ewe body weight (BW). Ewes supplemented with 1% PUFA were heavier than ewes supplemented with 0 or 2% (94.8 vs 91.0 and 89.8 ± 1.06, respectively). There were no differences in BCS, milk production, fat or protein concentration, but there was a linear increase (linear $P = 0.06$) in lactose concentration. There was no difference in lamb BW, or plasma glucose and NEFA concentrations ($P > 0.1$). However, there was a time by treatment interaction for average daily gain (ADG; $P < 0.05$). Lambs of ewes supplemented with PUFA at 1% showed a higher ADG (0.36 kg/d) than the 0% (0.31 kg/d) or 2% (0.33 kg/d) supplementation groups. The ADG from d 15 to d 60 was similar for the three

treatments. A linear ($P = 0.04$) decrease in mRNA concentration of diacylglycerol acyltransferase 1, and quadratic changes ($P < 0.06$) in fatty acid binding protein 4, fatty acid transport protein 1, and peroxisome proliferator-activated receptor α were observed. This suggests that the supplementation of EPA and DHA during gestation affects ewe and lamb growth, and these effects may be dose dependent. The fact that the 1% EPA and DHA supplement showed a heavier BW for the ewes and the highest ADG for the lambs without affecting in the same manner MY or composition creates more questions to be answered regarding the biological effects of PUFA. Some of these performance changes could be attributed to changes in gene expression.

Introduction

Producers are always looking for ways to improve growth rates and overall performance of their livestock. Studies in livestock have shown that polyunsaturated fatty acids (PUFAs) are effective in improving biological performance (Lopes et al., 2009). However, PUFA absorption in ruminants is difficult because rumen microbes biohydrogenate these PUFAs before they are able to make it to the small intestine for absorption (Wachira et al., 2000). To avoid this problem Ca salts of PUFAs have been developed with different sources of fatty acids (Grummer, 1988). Despite the known function of the lipids as an energy source, PUFAs have been known to affect gene expression in lipolytic and lipogenic enzymes (Clark, 2001). Despite how much is known about the role of fatty acids in performance, there is little information on the effect of PUFAs as modifiers of fetal programing. Fetal programing refers to the fact that changes that occur in a dam during gestation may have an impact on the offspring (Du et al., 2009). Current research (Coleman et al., 2018a) has shown that feeding 0.39% of a Ca salt rich in DHA and EPA, during the last 50 days of gestation in ewes, modifies milk fatty acid composition even 30 days after

supplementation has ended, without affecting ewe or lamb performance until weaning. The lack of difference in performance could correspond to a lack of physiological effect of the PUFAs at this time, or that the amount used was not enough to produce changes.

Based on the previous results, I hypothesize that EPA and DHA supplementation in late gestation has a dose dependent effect on ewes and their offspring performance and plasma metabolites, and on the ewe adipose tissue gene expression and performance. Therefore, the overall goal of this study was to determine if the use of different concentrations of EPA and DHA in the diet will have significant effects on the ewe performance (growth and milk production) and on their lamb after birth and into its adult life.

Materials and Methods

This study was conducted at the Sheep Center of the Ohio Agricultural Research and Development Center, Wooster, Ohio (IACUC #2016A00000013). Seventy-two pregnant ewes were blocked by lambing dates and randomly assigned to the different treatments. The ewes were also blocked based on body condition score (BCS) and placed in pens (experimental units) of three ewes. Ewes were divided into three groups (n=24, 8 pens per group) which received either 0%, 1%, or 2% of an enriched diet of EPA and DHA (StrataG113, Virtus Nutrition LLC, Corcoran, Ca). These doses were selected based on previous results (Coleman et al., 2018a) in which a lower dose did not change offspring performance at weaning but did change adipose tissue gene expression of the ewes. Ewes received this supplement along with a diet (Table 1) that met the NRC (2007) requirements for ewes in late gestation. They began receiving the fatty acid supplement 50 days prior to expected lambing day. At day -20, a subcutaneous adipose tissue sample was taken on one ewe per pen, as described by Coleman et al. (2018b). At lambing

the diet was discontinued and all ewes and lambs were moved to a common pen, where they received the same diet.

Within 12 hours following lambing, the lambs were weighed and bled (taken from the jugular vein). The lambs remained with their lactating mothers until approximately 60 days of age (weaning). At 15 and 60 days of age the lambs were once again bled and weighed. The weights were calculated to measure average daily gain (ADG). On day 15 milk production on the ewes was recorded as described in Coleman et al., (2018b).

Adipose tissue samples were used to measure mRNA concentration of genes involved in lipolysis and lipogenesis (Table 2). The method used for this analysis was the NanoString technology as described previously (Coleman et al., 2018b).

Blood samples were centrifuged the day of collection, and plasma was separated, aliquoted, and stored at – 80 degrees C until analysis. Plasma obtained from the lambs at d 0, 15, and 60 was used to measure glucose (Relling et al., 2010) and NEFA concentration (Johnson and Peters, 1993).

Ewe performance data, plasma glucose and NEFA concentrations were analyzed as a randomized complete block design with a repeated measurement procedure (SAS 9.4). The model considered the fixed effects of time, treatment and their interaction, and the random effect of ewes within the pens. For mRNA concentrations, a similar model was used without time and its interactions. As for the performance data of lambs, the model also included sex of the lambs and number of lambs at lambing (single, twin or triplets), as well as the actual time of sampling related to birth day as co-variables, which were removed from the model if they were not significant ($P > 0.1$). Linear and quadratic polynomial contrasts were used for mean separation.

Results and Discussion

Dam performance

Ewe performance data in terms of body weight (BW) had a quadratic treatment effect ($P < 0.01$; Table 3). However, the effect was not as expected. Ewes supplemented with a 1% dry matter concentration of EPA and DHA had an average body weight of 89.8 kg. The ewes fed the 2% supplement weighed 91.0 kg and 0% weighed 94.8 kg. Not only did BW decrease with increasing concentration, but the 1% supplement may have had more of an effect than the 2% concentration on the overall ewe BW. This may be explained by the adipose tissue gene expression data. As for BCS, no time by treatment effect was identified based on supplementation ($P > 0.1$; Table 3).

Subcutaneous adipose tissue

The genes used in the current study are involved in lipogenesis (Lipoprotein lipase, LPL; fatty acid synthase, FAS; diacylglycerol acyltransferases, DGAT 1 and 2), lipolysis (hormone sensitive lipase, HSL; adipose triglyceride lipase, ATGL) or fatty acids transport and signaling (fatty acid binding protein 4, FABP4; fatty acid transport protein 1, FATP1; and peroxisome proliferator-activated receptors α, β/ δ and γ). Subcutaneous adipose tissue gene expression exhibited linear and quadratic treatment effects ($P < 0.1$; Table 4). The mRNA concentration for DAGT1 had a linear decrease with the supplementation of EPA and DHA concentrations of 0%, 1%, and 2%. This shows that the expression of the gene was down-regulated as the concentration increased, with the exception of 1% and 2% matching the results found for ewe body weight. As DAGT1 mRNA concentrations decreased, most likely so did translation of the enzyme DAGT1, meaning less formation of triglycerides (Lui et al., 2011). This may suggest that the ewe does not need to synthesis tryglicerides in the adipose tissue. This also suggests that 2% of an enriched

source of EPA and DHA may be too high of a dosage. There was no effect on the mRNA concentration of LPL, FAS, DAGT2, HSL or ATGL. The concentration of mRNA for FATP1 had quadratic treatment effects ($P = 0.06$; Table 4), where ewes supplemented with 0% (80.2) or 2% (55.6) had a greater concentration than the ewes that received 1% (44.0). The pattern of mRNA concentration for FATP1 is similar to what was shown for DAGT1, which could imply that the transport of the fatty acid might be decreased, which downregulated the gene expression for the synthesis of new triglycerides. Past studies have shown that the expression of FATP1 mRNA concentration changes in the placenta of bovine (Desantadina et al., 2018) due to physiological stage. Therefore, it is possible that this might be responsible for the mobilization of specific fatty acids to the fetus. However, the present study does not allow us to confirm that assumption. Also, the current data do not provide details of why the 1% supplemented fat would down-regulate the gene expression more than 2%. For mRNA of FABP4 and PPARα, there was a significant quadratic treatment effect ($P < 0.05$; Table 4). The receptor gene, FABP4, followed the increasing concentration of 0% (76089), 2% (126267.5), and 1% (171332.5). The gene FABP4 is involved in binding fatty acids to allow movement within the adipose cell (Coleman et al., 2018b). This would explain why the 1% value was higher than the 0% value. If the ewe is receiving more supplement and growing faster, she would be better utilizing fatty acids. This, again, does not explain why the 2% supplementation data would be lower than the 1% data. The only assumption that can be made as of now is that the 2% EPA and DHA supplement may have contained too much PUFA and resulted in fatty acids staying in storage. PPARα, which is involved in lipogenesis, again showed 1% as the lowest concentration amount of mRNA.

Milk production and composition

Milk production, fat (%) and protein (%) were not affected by the different concentrations of EPA and DHA in the diet ($P > 0.6$), but there was a linear increase in lactose concentration ($P = 0.01$; Table 5). The change in lactose concentration did not change the total energy produced per kilogram of milk or during the 3-hour period in which milking took place ($P > 0.1$; Table 5). The milk production data do not correlate with the lamb ADG data, in which lambs born to ewes supplemented with 1% EPA and DHA had greater ADG than the other 2 treatments, but that difference was not due to an increase in milk production or energy by the ewe. Therefore, something else other than energy intake modified the growth of the lambs born to ewes supplemented with EPA and DHA.

Lamb performance

Significant differences in lamb final body weight were observed ($P > 0.1$) (Table 6). However, when data were analyzed as ADG, there was a time by treatment interaction ($P < 0.01$). At day 15, lambs born to ewes supplemented with 1% PUFA had a higher ADG of 0.36 kg/d. The 0% supplementation had an ADG of 0.31 kg/d and the 2% group had an ADG of 0.33 kg/d. While there was a significant effect of supplementation on ADG from birth to 15 days of age, this again does not correspond with the hypothesis. It was hypothesized that, as concentration increased, so would ADG. However, 1% showed a higher ADG than the 2% supplementation. Average daily gain from day 15 to 60 did not show any difference due to supplementation. As described previously, the difference in ADG at day 15 was not associated with changes in milk yield or milk energy produced from the ewes.

As of now there is no information or data to explain why the 1% concentrated supplement of EPA and DHA is more significant than that of the 2% concentration. As discussed

with the ewe data, this result may suggest that the supplementation of 2% overloaded the capability of the animal. Further research should focus on the first 15 days postpartum. Also, more investigations should be done to examine why a smaller concentration of EPA and DHA in the diet would have a greater effect on lamb performance. The goal should be to find the optimum concentration to improve rate of gain. It is worth mentioning that these lambs had a different outcome of performance after weaning (Nickles et al., 2018). There was a linear increase in BW ($P < 0.01$) and a quadratic response for ADG ($P < 0.08$). Lambs born from 0 and 1 % PUFA had similar ADG, whereas lambs born to ewes supplemented with 2 % PUFA had a 10 % increase in ADG compared with lambs born to ewes supplemented with 0 and 1 % PUFA (Nickles et al., 2018).

Glucose and NEFA concentrations also did not show any significant differences corresponding with the EPA and DHA supplementation of 0%, 1% and 2% ($P > 0.1$) (Table 6). These results are also dependent on many factors. However, in this scenario we must assume that this is because the supplementation did not have an effect on plasma metabolites.

In conclusion, ewe supplementation in late gestation with different doses of EPA and DHA had an impact on ewe and lamb performance in a nonlinear fashion. It decreases ewe BW at an inclusion rate of 1% of the enriched source of PUFA, but not at a rate of 2%. The change in performance is not associated with changes in adipose tissue gene expression despite the fact that DAGT1, FABP-4, FATP1 and PPAR α are changed. However, there was a time by dose fed treatment effect on lamb ADG from birth to weaning. This effect was temporary, and the weaning BW was similar for all treatment groups.

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Tables

Table 1: Diet composition (% DM basis) of diets containing 0, 1 or 2 % of supplemented enriched sources of EPA and DHA, fed to pregnant ewes at 2 kg/d during the last 50 d of gestation

Ingredients	Diet composition (% of DM bases)		
	0%	1%	2%
Corn Silage	30.54	30.54	30.54
Alfalfa haylage	17.96	17.96	17.96
Ground corn	10.10	8.89	7.97
Soy hulls	30.65	30.88	30.81
DDGS[1]	0.44	0.48	0.48
limestone	10.10	10.07	10.04
Mineral supplement[2]	0.20	0.18	0.18
Ca salt supplement[3]		1.01	2.03

[1]DDGS= Distiller's dried grains with solubles.
[2]Vitaferm Concept-Aid Sheep (BioZyme, St. Joeseph, MO). Contains 15.5% Ca, 5% P, 16% NaCl, 4% Mg, 2% K, 10 ppm Co, 70 ppm I, 2850 ppm Mn, 16.4 ppm Se, 2500 ppm Zn, 130000 IU/kg vitamin A, 7500 IU/kg vitamin D_3, 550 IU/kg vitamin E.
[3]Strata G113 Virturs Nutrition

Table 2. Gene names and GenBank accession numbers

Gene Name[1]	Accession Number
LPL	NM_001009394.1
ATGL	NM_001308576.1
HSL	NM_001128154.1
DGAT1	NM_001110164.1
DGAT2	XM_012096078.2
FABP4	NM_001114667.1
FAS	XM_015098375.1
FATP1	XM_015095580.1
PPAR alpha	XM_012175774.2
PPAR beta/delta	XM_004018768.3
PPAR gamma	NM_001100921.1
Beta-actin	NM_001009784.1
Beta-2 microglobulin	NM_001009284.2
Ciclophilin A	NM_001308578.1
GAPDH	NM_001190390.1
PGK1	NM_001142516.1

[1] LPL = lipoprotein lipase; ATGL = adipose triglyceride lipase; HSL = hormone sensitive lipase; DGAT1 = diacylglycerol acyltransferase 1; DGAT2 = diacylglycerol acyltransferase 2; SCD = stearoyl-CoA desaturase; ELOVL2 = elongation of very long chain fatty acid 2; ELVL4 = elongation of very long chain fatty acid 4; ELOVL5 = elongation of very long chain fatty acid 5; FABP4 = fatty acid binding protein 4; FAS = fatty acid synthase; FATP1 = fatty acid transport protein 1; GIP = glucose-dependent insulinotropic polypeptide; RXR = retinoid X receptor; Cox-2 = cyclooxygenase 2; 5-lox = 5-lipoxygenase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PGK1 = phosphoglycerate kinase

Table 3: Body weight (BW) and body condition score (BCS) of ewes supplemented with 0, 1, or 2 % EPA and DHA enriched diet during the last 50 days of gestation

Item	Treatment[1]			SEM	P value	
	0	1	2		Linear	Quadratic
BW, kg	94.8	89.8	91.0	1.06	0.25	< 0.01
BCS	3.2	3.3	3.3	0.14	0.77	0.70

[1] Treatments on ewes supplemented with 0, 1, or 2 % EPA and DHA enriched diet during the last 50 days of gestation; Body Condition Score measured on a scale of 1-5

Table 4: Concentration of mRNA from the subcutaneous adipose tissue obtained from late gestation ewes after 30 days of supplementation with 0, 1, or 2 % EPA and DHA enriched diets

Item[1]	Trteatments[2]			SEM	Treatment	
	0	1	2		Linear	Quadratic
LPL	3495	5406	2831	1428	0.75	0.23
FAS	8325	12347	14159	4220	0.26	0.80
DAGT1	1162	610	695	176	0.04	0.08
DAGT2	2705	7177	6172	2069	0.20	0.23
HSL	5041	4643	3831	731	0.13	0.76
ATGL	1502	1446	1211	217	0.24	0.67
FABP4	76089	171332	126267	16074	0.62	0.05
FATP1	80.2	44.0	55.6	12.6	0.09	0.06
PPAR α	171	149	194	16	0.16	0.02
PPAR β/δ	116	93	94	15	0.24	0.44
PPAR γ	2107	2284	2126	238	0.95	0.55

[1] Gene expression is a relative value estimated using the mean of beta-actin, beta-2 microglobulin, ciclophilin A, GAPDH and PGK1. LPL = lipoprotein lipase; FAS = fatty acid synthase; DGAT1 = diacylglycerol acyltransferase 1; DGAT2 = diacylglycerol acyltransferase 2; HSL = hormone sensitive lipase; ATGL = adipose triglyceride lipase; FABP4 = fatty acid binding protein 4; FATP1 = fatty acid transport protein 1; PPAR = peroxisome proliferator-activated receptors.

[2] Treatments on ewes supplemented with 0, 1, or 2 % EPA and DHA enriched diet

Table 5. Milk production and milk composition on d 15 post lambing of ewes supplemented with 0, 1 or 2 % of Ca salts containing EPA and DHA during the last 50 days of gestation

Item	Treatment[1]			SEM	P value	
	0	1	2		Linear	Quadratic
Milk yield, mL 3 hrs	294.9	299.1	307.8	24.93	0.75	0.94
Fat, %	8.1	7.8	7.8	0.38	0.66	0.74
Protein, %	4.1	3.9	4.1	0.10	0.88	0.20
Lactose, %	4.87	5.02	5.05	0.047	0.01	0.27
Total solids, %	5.776	5.96	6.00	0.059	0.02	0.29
MUN, %	14.75	13.75	13.32	1.341	0.51	0.87
Milk Energy, Mcal/kg	4.862	4.756	4.803	0.151	0.79	0.66
Milk energy, Mcal in 3 hrs	0.343	0.340	0.353	0.035	0.852	0.86

[1] Treatments on ewes supplemented with 0, 1, or 2 % EPA and DHA enriched diet during the last 50 days of gestation

Table 6: Body weight (BW), average daily gain (ADG), and plasma glucose and NEFA concentration of lambs born to ewes that received 0, 1, or 2 % EPA and DHA enriched diet during the last 50 days of gestation

Items	Treatment[1]			SEM	P value	
	0	1	2		Time	Time x treatment
BW, kg	14.6	14.7	14.6	0.49	0.95	0.88
ADG, d15, kg/d	0.31	0.36	0.33	0.109	0.10	<0.01
ADG, d30, kg/d	0.25	0.25	0.25			
Glucose	98.55	98.41	102.44	3.00	0.56	0.17
NEFA	581.0	514.4	566.0	52.78	0.63	0.86

[1] Treatments on ewes supplemented with 0, 1, or 2 % EPA and DHA enriched diet during the last 50 days of gestation; Average Daily Gain found from day 0-15, and day 0-30